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TISSUE MECHANISM ON ADAPTATION OF ANIMALS TO A REDUCED OXYGEN CONTENT IN THE ENVIRONMENT

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TISSUE MECHANISM OF ADAPTATION OF ANIMALS TO A REDUCED OXYGEN CONTENT IN THE ENVIRONMENT

[Following is a translation of an article by N. A. Verzhbinskaya in Izvestiya Akademii Nauk SSSR (News of the Academy of Sciences USSR), No. 3, Moscow, 1962, pp. 430-442.]

So far, it is far from clear which physiological and biochemical mechanisms provide for the acclimatization of animals to hypoxia, in what sequence they are included in the total combination of adaptive reactions of the body in response to the prolonged effect of a hypoxic medium, and which physiological and biochemical changes in the body should be regarded as an index of true or complete acclimatization to hypoxia.

The extensive literature dealing with the effect of various forms of hypoxia on the functions of animal organisms contains a tremendous number of facts depicting various fragments of the acclimatization process, which, however, cannot as yet be reduced to a single definitive concept of it.

For the purpose of putting order into the existing extensive material it is essential first of all to select for analysis only data pertaining to true acclimatization of animals to hypoxia. Following Ye. M. Kreps (1956), we call the totality of adaptive physiological and biochemical changes occurring in response to a prolonged effect of an altered environment and creating a new physiological condition of the body which is better adapted to the altered existential conditions "acclimatization to hypoxia." Not only good survival of organisms in the altered medium but, of necessity, also their normal multiplication, with good survival, normal development, and maturation of the progeny should also be considered an index of complete acclimatization.

It would be no exaggeration to state that at the present time the role of tissue adaptation to hypoxia during the acclimatization process has been least clarified.

The main cause of variegation of the experimental data is the extreme variety of the experimental conditions. In the great majority of studies the oxygen content in the medium was lower than that in which true acclimatization is possible. The experience of Ye. M. Kreps' laboratory showed that a slight reduction of the oxygen content in the atmosphere of the chamber, below ten percent (at sea level), can interfere with the reproductive function of rats--animals which are very resistant to hypoxia (Kreps and others, 1956a).

In many studies in which changes in the activity of

tissue oxidative systems have been described only the good survival of animals under hypoxic conditions and the development of a number of physiological changes in them have been noted; no study has been made of the multiplication of animals and, therefore, there has not been adequate basis for assuming the development of true acclimatization in the sense in which it is presented here. In a number of studies a more or less significant increase of activity of the main cell oxidative systems, determinable under in vitro conditions, has been established. Z. I. Barbasheva (1952, 1958) described an increase in the activity of brain cytochrome oxidase and that of other tissues, a slight increase in the cytochrome C content in the muscles and other changes in the tissues of mice and rats trained for hypoxia for a month. More marked changes under conditions of more severe but briefer hypoxia were observed by Z. K. Sulimo-Samuyllo (1952). Delachaux and Tissieres (1956), Delachaux and Berson (1947), Harnischfeger and Opitz (1950) found a considerable increase in the content of cytochrome C in the muscles of rabbits and guinea pigs exposed to the effect of hypoxia in different forms of experiments. Delachaux and Tissieres also found a considerable increase in the myoglobin content of the muscles. In other works these data have not been confirmed (Kreps and others, 1956a, b, c). Under similar experimental conditions which frequently accurately copied those described, the authors did not find any increase in enzyme activity, essential changes in the cytochrome C content of the brain, muscles or other tissues, or in the myoglobin content in the muscles of animals trained for hypoxia.

In the group of works which made a study of changes in tissue metabolism occurring under conditions of a known severe hypoxia incompatible with acclimatization, a distinct reduction of activity of oxidative enzymes, a reduction in the rate of turnover of phosphorus-containing compounds were always found (Kreps, Smirnov, Chetverikov, 1954; Shapot and Gromova, 1954; Domontovich, 1958); preliminary training of the animals under conditions of moderate hypoxia reduced the magnitudes of the changes which followed (Domontovich, 1958).

In previous studies of Kreps' laboratory (Kreps and others, 1956a, b, c; Kreps, 1956; Voytkevich, 1958) the results of a study of the acclimatization process of animals to hypoxia were presented under conditions of a long (nine years now) chronic experiment. In the first works data were presented on the condition of oxidative tissue metabolism in the first four generations of rats acclimatized in an atmosphere with an oxygen content reduced to 10.5 percent at normal pressure. However, at that time, in the fourth generation, the multiplication of rats was considerably retarded; the conditions of the chamber proved to be too severe, and the hypoxia was incompatible with the prolonged existence of the animals

over a number of generations. After a slight lessening of the degree of hypoxia (the oxygen content was raised to 11 percent) multiplication was restored, and at the present time we have 12 generations of rats which have been born, bred and have given progeny under conditions of oxygen deficiency.

In the first works it was shown that the activity of the main cellular oxidative enzyme systems did not undergo any appreciable changes in any of the tissues studied. However, at that time a change was found in the properties of the cytochrome system in the brain and cardiac muscle of "hypoxic" rats.

The activity of the cellular oxidative enzyme systems was determined under in vitro conditions at that time. In this form of experiment it is difficult to select the conditions which recreate the physiological level of tissue metabolism characteristic of it in vivo. Under in vitro conditions the potential activity of enzyme systems determining the maximum capacity of the system within limits of which the physiological variations in its activity were carried out was measured. Considerably better suited to investigations of this kind is the form of experiment on the intact, uninjured organism with its operating regulatory systems, with a normal cell concentration of all the agents which determine the rates of the enzymic reactions.

In the present work, which in a chronic experiment continues the investigation of the acclimatization process of animals to hypoxia (Kreps 1956), the results of a study of the rate of turnover of adenosinetriphosphoric acid (ATP) in the brain in vivo and the permeability of the hematoencephalic barrier in 11 generations of rats born, bred and reproducing in a hypoxic medium containing 10.5-11 percent oxygen are presented. The conditions under which the rats were kept have already been described in detail (Kreps and others, 1956a). White rats were kept in a gas flow chamber, in which for 12 hours a day a mixture of air and nitrogen was administered; the carbon dioxide and moisture in the chamber were absorbed by soda lime and silica gel. In the present work, for technical reasons, it was impossible to study all 11 generations of rats; the first and second generations were investigated and then, from the eighth through the 11th. The generations between the second and eighth were not studied.

Method

In this work the method of determining the turnover of ATP in the brains of warm-blooded animals which we worked out (Verzhbinskaya, 1957, a, b, 1958) was used. The method is based on the utilization of small doses of radioactive phosphorus injected into the animals for very short times,

five, ten and 15 minutes, with the obligatory determination of the blood content of the brain and the introduction of a corrective factor for the radioactivity of cerebral blood. In every experiment four rats were investigated (two "hypoxic" and two control rats). The animals were injected intraperitoneally with Na_2HPO_4 labeled for P in a quantity of 0.2 microcourie of P^{32}+10 micrograms of P^{31} per gram of body weight in a volume of 0.5 cc per rat. After five, ten and 15 minutes in various experiments and 60 minutes after the injection of the labeled phosphate the animals were frozen in liquid oxygen. At the last moment before immersing the animal's head in the liquid oxygen an incision was made in the neck through the carotid artery, and several drops of blood were collected in liquid oxygen. By this method simultaneous fixation of the blood and brain was achieved, which is very important in experiments of short duration. The stony-hard brain was cut out of the skull and ground up by means of liquid oxygen into a fine powder, the bulk of which was transferred to previously weighed flasks with half-frozen five-percent trichloracetic acid. In the small flask containing ten-percent trichloracetic acid a certain quantity of frozen blood was taken up, and, finally, a small quantity of brain powder (25-60 milligrams) was put in a weighed flask containing 0.2 percent NaCl solution. The portion of brain powder put in the five percent trichloracetic acid was used for the determination of the content and radioactivity of inorganic phosphate of the brain (IP), of brain adenosinetriphosphate (ATP) and brain creatine phosphate (CP); the portion of brain powder put into the hypotonic NaCl solution was used for a determination of the content of blood in the brain and for introducing a corrective factor for the radioactivity of blood phosphates; and, finally, in blood put into the ten-percent trichloracetic acid a determination was made of the content and radioactivity of IP and ATP of the blood.

The content of P in fractions of IP and ATP of the brain and blood was determined in isobutanol extracts of an IP precipitate by the Delore method and in isobutanol extracts of a mercurial precipitate of ATP-ADP after a ten-minute hydrolysis of it in 1 N HCl at 100°. Phosphomolybdate was reduced with $SnCl_2$ according to the A. V. Kotel'nikova method (1957). Control tests were also performed in which the phosphomolybdate was extracted with isobutanol in a reduced form by the Fiske and Subbarow method.

The radioactivity of isobutanol extracts was measured on a T-25-BFL end-type counter and a type B scaler.

The figures obtained in the experiments contained all the necessary data for calculation of the specific radioactivity (SR), corrected for the radioactivity of blood present in the brain and in the IP and ATP fractions of the

brain. Then, the RSR [relative specific radioactivity] of the brain ATP was calculated, which in percentages expressed the ratio $\frac{\text{SR-ATP}}{\text{SR-brain IP}}$. The radioactivity of the CP fraction of the brain was used for a more complete record of radioactivity passing from the inorganic phosphate fraction of the brain into the organic phosphate fraction of the brain. Considering that only ADP is the primary acceptor of the labeled P^{32} during the course of oxidative phosphorylation and that the radioactivity in CP can occur only as the result of interesterification of CP and ATP, we found it more accurate to refer the entire radioactivity measured in fractions of ATP and CP of the brain to the P content in ATP alone; in this way the SR of ATP was calculated, which includes the entire radioactivity of the labile macroergic adenylic nucleotides of the brain. In a corresponding manner, the RSR of ATP was calculated, which, according to our concept, more fully reflects the rate of the oxidative phosphorylation process in the brain.

The ratio $\frac{\text{SR-IP of brain}}{\text{SR-IP of blood}}$ gave the RSR of IP of the brain and characterized the permeability of the hematoencephalic barrier to P.

The admixture of blood in the brain was determined by the benzidine method, worked out for quantitative determination of blood hemoglobin by Bing and Baker (1931, 1932) and adapted by us for a determination of the blood content in the tissues. Small portions of the brain powder with 0.2 percent NaCl and portions of blood were weighed on an analytical balance and diluted with two percent NaCl to 1:400, shaken vigorously, and left in the refrigerator until the next day. In the morning the tests were centrifuged (ten minutes at 3000-4000 rpm), and the centrifugates were used for further determination.

A color calibration scale of blood was made for the brain extracts. For this purpose the blood was first diluted with NaCl to 1:2000, 1:4000 and 1:8000, and then each dilution mentioned was again diluted by five times with one of the brain extracts. By this method a dilution series of blood was obtained of 1:10,000, 1:20,000 and 1:40,000, in which four-fifths of the volume was constituted by the brain extract and one-fifth of the volume by a salt solution of blood hemoglobin.

Into a number of test-tubes, each containing 0.5 cc of the benzidine reagent, 0.5 cc of each blood dilution (in two parallel tests) was poured; two blank tests were performed, each containing 0.5 cc of benzidine reagent, 0.4 cc of brain extract and 0.1 cc of 0.2 percent NaCl. The test-tubes were carefully shaken, and 0.75 percent H_2O_2 was added in a quantity of 0.25 cc to each. Color developed in two

hours, changing from a transient blue to a persistent red. After two hours, 20 percent-acetic acid was added to all the test-tubes to a final volume of 5 cc, mixed, and examined colorimetrically after eight minutes on a photoelectric FEK-M colorimeter against a blank test which contained a mixture of all the components with the exception of H_2O_2. A calibration curve was constructed as follows: on the abscissa axis the extinction of decreasing blood dilutions was plotted; on the ordinate axis, the percentage of blood in the brain. The brain samples were treated simultaneously with the blood samples and were examined colorimetrically against a corresponding blank test. Correspondence of the brain color in a dilution of 1:400 with the color of blood in dilutions of 1:10,000, 1:20,000 or 1:40,000 corresponds to a 1-, 2-, or 4 % blood content in the brain. The intermediate blood concentrations were found from the curve.

The benzidine reagent was prepared from recrystallized benzidine according to the method of the authors.

Results of the Experiments

In Table 1 the arithmetic means and the mean errors of the values for the SR and RSR of ATP and IP of the brain and the values of the SR of IP of blood in control rats and in rats acclimatized to hypoxia are shown five and 15 minutes after they had been injected with radioactive phosphate.

By analyzing the first two horizontal columns in the Table, which contain the data of five-minute experiments for control rats and those acclimatized to hypoxia, we can find a distinct, statistically significant increase in the renewal rate of brain ATP in the latter.

The turnover rate of ATP in brain metabolism, calculated according to the data of five-minute experiments (Verzhbinskaya, 1957b, 1958), is equal to the following in control rats: 60±5 micromoles of P of ATP per gram of brain per hour and, for the acclimatized rats, 85±9 micromoles of P of ATP.

The results of 15-minute experiments also show the considerably greater renewal rate of ATP* in the brains of acclimatized rats by comparison with the controls. From the data of 15-minute experiments it is impossible to calculate the renewal rate of ATP in the brain metabolism, because during this period the labile groups of ATP are recreated twice in control rats and three times in "hypoxic" rats during the metabolic process. These conditions are unsuitable for calculating the renewal rate of ATP in the process of oxidative metabolism. However, the fact of the higher renewal rate of ATP of the brains of acclimatized rats is also confirmed by the 15-minute exposure periods.

Table 1

Average Values for the Turnover Rate of Labile P of ATP in the Rat Brain

0.2 Microcuries of P^{32}+10 Micrograms of P^{31} Were Injected per Gram of Body Weight in a Volume of 0.5 cc per Rat.

1 Экспозиция	2 Фракция	3 мкг Р/1 г мозга, M±m	4 уд 1 мкг Р имп/мин, M±m	5 $УР^{M}$—АТФ % M±m	6 $УР^{K}$—НФ% M±m	n
7 Контроль 5 мин.	12 АТФ*	145±7,5	4,0 ±0,7	27,3±2,1	1,1±0,21	19
	НФ	131±8,2	15,4 ±3,1			
	Р крови	52±2,7	1570,0±166,0			
8 Гипоксия 5 мин.	12 АТФ*	158±8,2	4,7 ±0,59	35,4±4,0	2,1±0,46	22
	НФ	124±9,3	16,1 ±2,4			
	Р крови	51±2,8	1255,0 ±138,0			
9 Контроль 10 мин.	13 АТФ*	158	15,4 ±3,1	37,0±9		7
10 Контроль 15 мин.	12 АТФ*	155	10,6±1,6	1,4±3,8	1,4±0,18	19
	НФ	135	22,0±2,9			
	Р крови	56	1620,0±154,0			
11 Гипоксия 15 мин.	12 АТФ*	147	11,9 ±1,2	63,0±6,1	2,5±0,5	22
	НФ	130	24,6 ±4,5			
	Р крови	49	1219,0 ±79,0			

Note. *In the control rats the time needed for the turnover of the two terminal groups of labile P of ATP was equal to seven minutes. The rate of turnover of the two labile P groups of ATP was equal to 60 micromoles of P per gram of brain per hour. In hypoxic rats the time needed for the turnover of the two labile P groups of ATP was equal to five minutes. The rate of turnover was equal to 85 micromoles of P per gram of brain per hour. 1. Exposure time; 2. Fraction; 3. Micrograms of P per gram of brain, M±m; 4. SR of one microgram of P, pulses per minute, M±m; 5. RSR^{M}-ATP, %, M±m; 6. RSR^{K} of IP, %, M±m; 7. Control, 5 minutes; 8. Hypoxia, 5 minutes; 9. Control, 10 minutes; 10. Control, 15 minutes; 11. Hypoxia, 15 minutes; 12. ATP*, IP,, P of blood; 13. ATP*.

The ATP content in the brains of "hypoxic" and control rats, as is seen from the Table, is the same.

Along with an increase in the turnover rate of ATP in the metabolism of brains of rats acclimatized to hypoxia an increase is observed also in the rate of penetration of P^{32} through the hematoencephalic barrier. In the sixth vertical column of the Table, which contains the figures for the RSR-

IP of the brain, is seen that this figure is higher in rats acclimatized to hypoxia, which is evidence of the greater rate of penetration of P^{32} from the blood into the brains of "hypoxic" rats than in the controls. This fact has already been described (Smirnov and Chetverikov, 1953).

The interpretation of this fact offers certain difficulties. The high figure for the RSR-IP of the brains of "hypoxic" rats means that a larger quantity of P^{32} passed from the blood stream into the brain parenchyma per unit time in the "hypoxic" rats than in the controls. This change may have been caused by an increase in the permeability of the hematoencephalic barrier or by an increase in the contact surface of brain tissue with the capillary network.

According to the data of V. I. Voytkevich (1958), in several generations of "hypoxic" rats an increase in the amount of blood in the brain was observed by 43-46 percent, caused both by a dilatation and a hyperplasia of the cerebral blood vessels. Hence it follows that in the "hypoxic" rats there is an increase in the surface through which an exchange can occur between blood and brain. However, there is a reason to believe that in the "hypoxic" rats a direct increase in the permeability of the hematoencephalic barrier is also observed. Thus, in all the "hypoxic" rats which we investigated the radioactivity of the blood five and 15 minutes after the injection of the same quantity of P^{32} was less than in the controls. This was probably caused by the greater resorption rate of P by all the body tissues in the "hypoxic" rats and, it must be considered, is evidence of a general increase in the permeability of barriers in the body, particularly, an increase in the permeability of the hematoencephalic barrier.

In addition, getting ahead of ourselves, it may be noted that in the late generations of "hypoxic" rats the blood content of the brain remains increased, and the permeability of the hematoencephalic barrier returns to normal. A comparison of all the facts observed causes us to conclude that an increase in the rate of penetration of P^{32} through the hematoencephalic barrier in "hypoxic" rats is in some measure due to an increased permeability of the barrier.

As further analysis shows, however, the relationship between the rate of oxidative phosphorylation and the rate of penetration of P^{32} through the hematoencephalic barrier is very complicated.

In Fig. 2 curves are presented which show the statistical distribution of the values of RSR of ATP of brain according to the data of various experiments. The curves were constructed in the following way: the entire range of individual variations of the values of RSR-ATP of the brain was divided into different groups: 0-20; 20-40; 40-60; 60-80%

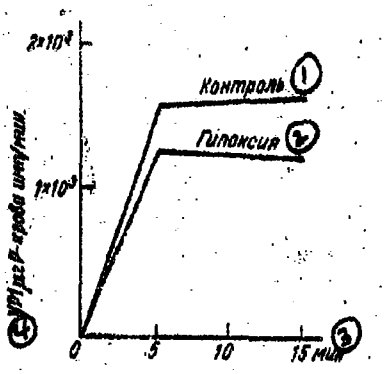

Fig. 1. Change in the Average Figures for the Specific Radioactivity of IP of Blood of Control and "Hypoxic" Rats with Time, Pulses per Minute per Microgram of P. 1. Control; 2. Hypoxia; 3. Minutes; 4. Specific Radioactivity of One Microgram of P in the Blood in Pulses per Minute.

plotted on the abscissa axis; the number of experiments performed for each group was expressed in percentages of the total number of experiments and was plotted on the ordinate axis. The bottom curve, a, which depicts the statistical distribution of data for the control rats, has a single peak with a maximum in the region of values of RSR-ATP=20-40. Curve b, which represents the distribution of RSR-ATP of the brain values for all "hypoxic" rats investigated, has a more spread-out shape with a second maximum noted, which attests to the occurrence of inhomogeneity in the experimental material, of the occurrence of a certain number of individuals with a higher rate of oxidative phosphorylation in the brain. First, we regarded this curve as an index of individual inhomogeneity of the material, an index of the fact that during the process of acclimatization to hypoxia various individuals show a greater rate of development of the adaptive changes. However, further analysis shows that in the first and second generations of "hypoxic" rats (curve c) the statistical distribution of the values for RSR-ATP of the brain completely coincides with the controls--the curve has a single maximum in the region of 20-40 and falls uniformly on both sides. In contrast to this, in the eighth to 11th generations of "hypoxic" rats the curve of statistical distribution of the values of RSR-ATP is very much different from the control--the first maximum in the region of 20-40 is reduced, and a second maximum is distinctly seen in the 60-80 region.

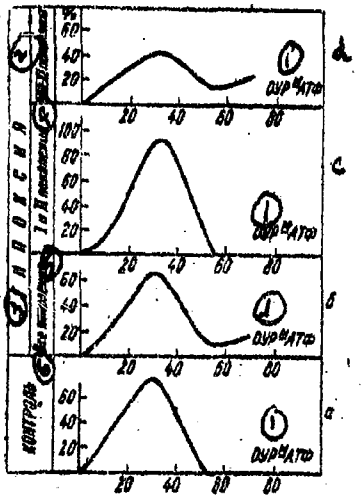

Fig. 2. Curves of Statistical Distribution of the Values of RSR-ATP of Brains of Control and "Hypoxic" Rats. a--Control Rats; b--All the "Hypoxic" Rats Investigated; c--First and Second Generations of "Hypoxic" Rats; d--Eighth-11th Generations of "Hypoxic" Rats. 1. RSR^{M} of ATP [the Superscript M Means in the Brain; the Superscript K Means in the Blood]; 2. Eighth-11th Generations; 3. Hypoxia; 4. First and Second Generations; 5. All Generations; 6. Control.

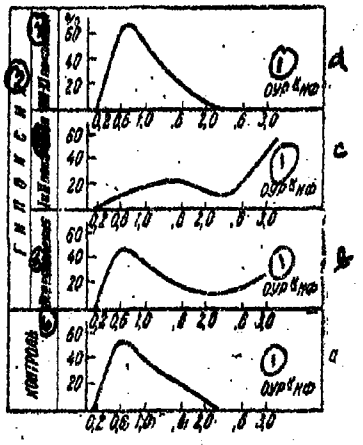

Fig. 3. Curves of Statistical Distribution of the Values of RSR-IP of the Brains of Control and "Hypoxic" Rats. a--Control Rats; b--All the "Hypoxic" Rats Investigated; c--First and Second Generations of "Hypoxic" Rats; d--Eighth-11th Generations of "Hypoxic" Rats. Explanations in Text. 1. RSR^{K}-IP [This is in error, the Superscript should be M]; 2. Eighth-11th Generations; 3. Hypoxia; 4. First and Second Generations; 5. All Generations; 6. Control.

We see that the change in the rate of oxidative phosphorylation in the brains of "hypoxic" rats is observed only in the later generations, eighth-11th in our experiments, and is absent from rats which have recently been placed in a hypoxic medium. The reaction to hypoxia on the part of cell metabolism is elaborated slowly, only over a number of generations of animals living under conditions of oxygen deficiency.

In Fig. 3 curves showing the statistical distribution of the values of RSR-IP of the brain are presented char-

acterizing the rate of penetration of P^{32} through the hematoencephalic barrier. The entire range of variations in the values of RSR-IP of brain has been divided into different groups: 0.2-1.0; 1.0-1.8; 1.8-2.6; higher than 2.6.

Control rats (curve a) are distributed according to a single-peak curve with a maximum in the region of RSR-IP values of 0.2-1.0.

Curve b, which depicts the statistical distribution of values of RSR-IP of the brains of all the acclimatized rats investigated shows a distinct double-peak character and a very much spread-out form. Again, now with regard to the barrier function of the brain, the inhomogeneity of the experimental material was expressed. Fifty percent of the individuals studied showed a very high rate of penetration of P^{32} from the blood stream into the brain (the second maximum in curve b). At the same time, fifty percent of the investigated individuals maintained the normal penetration rate of P^{32} from the blood into the brain characteristic of the control rats (the first maximum in curve b).

Analysis shows that all the changes in the condition of permeability of the hematoencephalic barrier with regard to P, come about in the first and second generations of "hypoxic" rats. The curve of statistical distribution of the values of RSR-IP of the brains of those generations (Fig. 3,c) has two maxima, shifted to the right compared with the control. The first, lower maximum occurs in the region of 1.0-1.8; the second, the main maximum, comes about in the region of figures above 2.6. The shift in the direction of an increase in the rate of P^{32} penetration through the hematoencephalic barrier is unusually distinct.

In the eighth-11th generations of "hypoxic" rats (Fig. 3,d) the statistical distribution of the figures for RSR-IP of the brain returns to the control level. Curve d has a single peak, has a maximum in the region of 0.2-1.0 and drops to 0 in the same region in which the control curve falls to 0. The rate of P^{32} penetration through the hematoencephalic barrier is normalized in those generations in which a reaction has already been elaborated to hypoxia by cell metabolism.

As the result of such an analysis by means of statistical distribution curves of the values of RSR-ATP of the brain and the values of RSR-IP of the brain it was made clear that in the early generations of rats exposed to the effect of a hypoxic medium (the first and second generations in our experiments) there is no reaction to hypoxia on the part of cell metabolism and there is a marked increase in the rate of P^{32} penetration through the hematoencephalic barrier. Later generations of rats, exposed to the effect of oxygen deficiency, the eighth through the 11th generations, show a re-

encephalic barrier, observed during the first period of acclimatization, is of biologic value. A positive result in the series of experiments undertaken would make it possible to consider the increased permeability of the hematoencephalic barrier in the first period of acclimatization an active adaptive reaction to hypoxia.

In the literature it has been firmly established that the normal accomplishment of the barrier function in the brain is maintained by the energy of oxidative metabolism. Any more or less marked disturbance of it--anaerobiosis, hypoxia, disorder of cytochrome oxidase, restriction of the supply of substrates--hypoglycemia--all these effects are accompanied by an increase in the permeability of the hematoencephalic barrier to vital dyes, to P^{32} and other substances (Tschirgi, 1952; Hess, 1955; Bakay, 1957; Verzhbinskaya, 1957, 1958).

In a number of experiments control and "hypoxic" rats were injected with substances which increase the permeability of the hematoencephalic barrier to P^{32} in the same doses: pituitrin (0.1 cc intraperitoneally), histamine (0.5 milligram intravenously) and, finally, simply a large volume of hypotonic saline solution (5 cc of a 0.2 percent NaCl solution intraperitoneally). All these agents produced a considerable increase, by two or three times, in the RSR-IP of the brain, which signifies an increased rate of penetration of P^{32} through the barrier (Table 2). In all experiments of this series a considerable, 50 percent, reduction was observed in the rate of oxidative phosphorylation in the brain (see Tables 1 and 2).

Because of the inadequacy of our knowledge about the physiological mechanism of penetration of substances, particularly P^{32}, through the barrier between the blood and the brain, it is impossible to put trust in the fact that there is a reduction in the rate of oxidative phosphorylation under these conditions. There is reason to believe that this is only an apparent phenomenon. In connection with the problem being analyzed, it is important to note only that the initial increase in the permeability of the hematoencephalic barrier is not associated with an increased rate of oxidative phosphorylation, permeability changes of the barrier probably occur secondarily, as the result of changes in the rate of oxidative metabolism. This gives us the right to express the idea that the increased rate of penetration of P^{32} through the hematoencephalic barrier, observed during the first period of acclimatization of rats to hypoxia, should be considered an indication of a certain degree of incompleteness of the barrier function occurring as the result of a deficiency of the energy of oxidative metabolism of the brain during this period.

Table 2

Average Figures Characterizing the Metabolic Rate of Labile P of ATP in the Brains of Rats Which Have Been Subjected to Additional Influences

(1) Экспозиция в мин.	(2) Фракция	(3) мк Р/1 г мозга	(4) УР 1 мкг. Р· мин/мин $M \pm m$	(5) Оурм—АТФ % $M \pm m$	(6) Оурк—НФ % $M \pm m$	n	(7) Примечания
Контроль (8)	(9) АТФ*	152	0,1± 1,3	15,4±1,1	2,6±0,5	5	Питуитрин 0,1 мл на крысу внутрибрюшинно (13)
	НФ	100	38,3± 7,0				
	Р крови	47	1806,0±493,0				
Гипоксия (10)	АТФ*	130	5,7± 0,8	18,0±2,4	3,9±0,8	5	То же (14)
	НФ	84	35,4± 6,4				
	Р крови	49	1035,0±192,0				
Контроль (11)	АТФ*	154	6,5± 0,6	18,0±0,7	2,1±0,5	4	Гидратация: 5,0 мл 0,2% NaCl на крысу внутрибрюшинно (15)
	НФ	93	42,9± 2,1				
	Р крови	49	2027,0±211,0				
Гипоксия (10)	АТФ*	129	4,6± 1,2	10,0±3,0	2,0±0,4	3	То же (14)
	НФ	89	26,3± 0,9				
	Р крови	40	1088,0±278,0				
Контроль (11)	АТФ*	187	28,0	15,0	4,5	2	Гистамин 0,5 мг на крысу в хвостовую вену (16)
	НФ	120	186,0				
	Р крови	70	4008,0				
Экспозиция 15 мин. (12)							
Контроль (11)	АТФ*	154	17,6± 7,0	40,0±4,0	2,4±0,6	5	Питуитрин 0,1 мл на крысу (13)
	НФ	124	47,4± 17,5				
	Р крови	49	2788,0±107,9				
Гипоксия (10)	АТФ*	136	0,8± 2,1	37,0±5,1	5,8±1,4	5	То же (14)
	НФ	81	31,6± 0,7				
	Р крови	52	753,0±233,0				
Контроль (11)	АТФ*	158	12,6± 2,3	28,0±2,2	2,2±0,6	4	Гидратация, 5,0 мл 0,2% NaCl внутрибрюшинно (15)
	НФ	105	48,1± 12,5				
	Р крови	48	2230,0±260,0				
Гипоксия (10)	АТФ*	137	17,7± 1,6	39,0±7,1	3,5±0,7	3	То же (14)
	НФ	110	47,7± 4,2				
	Р крови	48	1460 ±182,0				
Контроль (11)	АТФ*	181	38,0	32,0	3,3	2	Гистамин 0,5 мг на крысу в хвостовую вену (16)
	НФ	108	105,0				
	Р крови	60	2990,0				

[Continued on next page]

[Table 2, continued from previous page]

1. Exposure Time, 5 Minutes; 2. Fraction; 3. Microcuries of P per Gram of Brain; 4. SR of One Microgram of P, Pulses per Minute, M±m; 5. RSR^{M}--ATP Percent, M±m; 6. RSR^{K}--IP Percent, M±m; 7. Notes; 8. Control; 9. ATP*, IP, P of Blood; 10. Hypoxia; 11. Control; 12. Exposure Time 15 Minutes; 13. Pituitrin, 0.1 cc per Rat Intraperitoneally; 14. Same; 15. Hydration: 5.0 cc of 0.2 Percent NaCl per Rat Intraperitoneally; 16. Histamine, 0.5 Milligram per Rat into the Caudal Vein.

Conclusions

The process of acclimatization of mammals to an oxygen deficiency takes place over a long time, over a number of generations of animals.

In the first period of acclimatization, which, according to the data of this work, lasted at least for the lifetimes of two generations of rats, the survival of the animals under conditions of oxygen deficiency is assured by the mobilization of different physiological reserves of the body. All the reactions of the organism observed during this period are directed at maintenance of a normal oxidation in the brain, the organ which depends most on the energy of oxidative metabolism. During this period the condition of the organism cannot be called good. It is at the boundary of irreversible changes. Maintenance of the normal oxidation level in the brain and in other vital organs is achieved by an increased consumption of oxidative energy by the body as a whole during this period. A vicious cycle is created, capable of leading to a disorder of the energy metabolism of the body.

The increased permeability of the hematoencephalic barrier and the absence of a reaction to hypoxia on the part of the cell metabolism in the brain can serve as one of the indications of the unfavorable, unbalanced condition of the body during the first period of acclimatization.

The period when the tissue reaction to hypoxia appears and the permeability of the hematoencephalic barrier returns to normal should be called the "second period of acclimatization," the period of true acclimatization. Through the material of the present work this period was found beginning with the eighth generation of rats living in a hypoxic medium. By this period the capacity of more efficient and economical utilization of the energy of oxidative metabolism is elaborated. The increased rate of oxidative phosphorylation in the brain tissues probably occurs because

of a greater conjugation of oxidation with phosphorylation. The increased efficiency of oxidative metabolism contributes to the normalization of the barrier function of the brain.

The results of the present investigation were obtained through a study of the oxidative phosphorylation in the brains of intact, uninjured animals existing normally and capable of multiplication. There were no disorders introduced by the in vitro experiment, no destruction of the tissue structure, no disorder of nervous or humoral regulation, etc. This increases the reliability of the results obtained.

Finally, it seems important to us to note that under experimental conditions throughout the lives of 11 generations of higher vertebrates a cumulative effect was found from generation to generation, that is, a hereditarily reinforced effect of an altered environment on the condition of the most important process of the energy balance--the process of oxidative phosphorylation in the brain tissues.

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